

Microbiology of Shell Disease: Environmental sources and diversity

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Objectives of the Research:

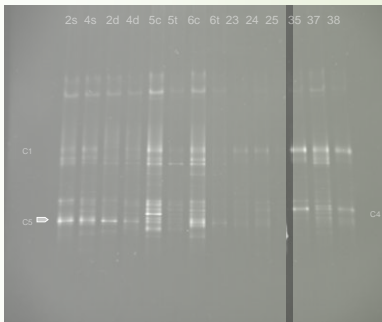
Our goal is to identify microorganisms involved in development of epizootic and impoundment shell disease for lobsters and other crustaceans. Shell disease could be caused by external invasion of the shell of certain bacteria. It would be important to show that these bacteria exist on all shell diseased lobsters regardless of area/time collected. However, it may be possible that an internal infection also exists in lobsters contributing to shell disease.

Results to Date:

1. Developed collection of samples from: *Macrobrachium ohione* (freshwater shrimp), *Callinectes sapidus* (blue crab), *Portunus gibbesii* (Iridescent swimming crab) and *Cancer irroratus* (rock crab). These have not yet been analyzed.
2. Results from prior work looking at epizootic shell disease in lobsters
 - Healthy lobsters have little or no bacteria present on shells. However not all healthy looking lobsters have sterile carapaces.
 - Belief that casual agent is a novel bacterium belonging to genus *Aquimarina spp* (*A. 'homaria'*).
 - A specific gamma or alphaproteobacterium is usually present in a consortium with *A. 'homaria'*.
 - Two eukaryotic organisms also found associated with shell disease: a nematode and a stramenopile (microscopic parasite).
 - No evidence on internal infection (2005) but newer paper by Bartlett et al in 2008, had bacteraemia prevalence between 15.2-88.3% depending on sampling location. Therefore want to look closer at bacteria in hemolymph.

Polymerase Chain Reaction (PCR) Denaturing Gradient Gel Electrophoresis (DGGE) Analysis. PCR-DGGE is a DNA fingerprinting technique. It is used to identify microorganisms based on their DNA.

PCR is a genetic approach that helps to create more copies of the part of the DNA that you want to examine (called amplification). These are usually sequences that are well conserved from organism to organism, for example sequences for the 16S rRNA gene. The 16S rRNA gene is commonly used tool for identifying bacteria.



A "fingerprint" of DNA on Denaturing Gradient Gel.

Once these extra copies of DNA segments are created, they are placed on a gel that has an electrical current passed through it. The DNA which has a negative charge will migrate towards the positive electrode. When it hits a certain part of the gel, the different base pairs of the DNA will be forced apart by the changing temperature and increasing gradient of denaturing chemicals in the gel. Different base pair sequences will denature or melt at different positions in the gel. The resulting pattern is called a "fingerprint".

To examine the types of bacteria in the hemolymph, we collected lobsters with shell disease from LIS, ME, RI, MA; one with impoundment disease and healthy lobsters from ME and Canada. After collection the hemolymph were plated on rabbit or sheep blood agar and used to isolate DNA for Denaturing Gradient Gel Electrophoresis (DGGE) analysis. Several 16S rDNA fragment bands were identified and sequenced (Figure 1). These were then matched against known bacteria in sequences and keyed out (Figure 2)

Figure 1

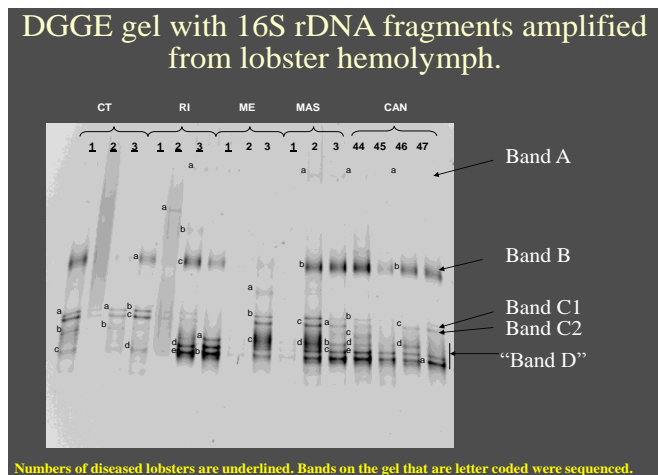


Figure 2

Closest relatives of bacteria commonly detected in lobster hemolymph		
DGGE band	Closest relatives	% of identity
Band A and B	Flavobacterial symbiont of ants	98-99%
Band C1	Uncultured Sphingobacteriales bacterium clone GASP-KA1W3_B02 16S ribosomal RNA gene, partial sequence.	95%
Band C2	<i>Ralstonia pickettii</i>	95%
“Band D”	<i>Delftia acidovorans</i> <i>Dechloromonas</i> sp. <i>Curvibacter gracilis</i> <i>Vibrio lentus</i> <i>Photobacterium profundum</i> <i>Pelobacter aquatica</i>	99% 99% 99% 100% 100% 99%

Summary:

- Bacteremia has a common occurrence in lobsters.
- Bacteria are found in hemolymph of both healthy lobsters and lobsters with two forms of shell disease.
- So far, no apparent correlation has been found between bacterial community composition and health status of an animal. However, *V. lentus* and *P. profundum* tend to be found in lobsters with shell disease, whereas *C. gracilis* and *Dechloromonas* sp. tend to be found in healthy lobsters.
- Bacteria detected in lobster hemolymph include (1) apparently symbiotic bacteria, (2) bacteria that may be associated with bacteremia (*P. profundum*, *C. gracilis*, *V. lentus*, *Ralstonia* sp., *Dechloromonas* sp., *D. acidovorans*), and (3) some bacteria only identified at higher taxonomic levels.

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